

Development and Characterization of a Gel Formulation Integrating Microencapsulated Nitrofurazone

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Abstract: Nitrofurazone (NTZ) is usually employed in the topical treatment of infected wounds and lesions of both skin and mucosa. Microencapsulation is a process utilized in the incorporation of active ingredients within polymers aiming at, among other objectives, the prolonged release of pharmaceutical compounds and protection from atmospheric agents (viz. moisture, light, heat and/or oxidation). With the goal of utilizing the microparticles containing encapsulated NTZ in pharmaceutical formulations, one prepared microparticles containing NTZ via ionotropic gelation of sodium alginate. The microparticles were characterized via scanning electron microscopy analyses, Fourier transform infrared spectroscopy (FTIR) analyses, via determination of encapsulation efficiency, and via thermal analyses (both TGA and DSC). The final gel formulation was also characterized rheologically. The extrusion/solidification technique employed to obtain the calcium alginate microparticles with encapsulated NTZ was found to be adequate, and produced an NTZ encapsulation efficiency of ca. $97.8\% \pm 1.1\%$. The calcium alginate microparticles thus obtained, with encapsulated NTZ, exhibited an oval shape and hydrodynamic diameters between 500 μm and 800 μm . From the thermal analyses performed, together with information from the infrared spectra, one may conclude that NTZ did not strongly bind to the polymer, which may be favorable for the release of the active ingredient. From the results obtained in the present research effort, one may conclude that the microparticles produced possess the potential to be utilized as carriers for NTZ in pharmaceutical formulations such as gels, ointments, and solutions.

Keywords: Antimicrobial topical applications, calcium alginate, ionotropic gelation, microencapsulation, microparticles, nitrofurazone.

1. INTRODUCTION

Nitrofurazone (NTZ) for human use is used for the topical treatment of infected wounds and injuries of the skin and mucous membranes. NTZ presents a broad spectrum of action, acting against various Gram-positive and Gram-negative microorganisms, but its mechanism of action is not fully understood. According to Ryan *et al.* [1], the mechanism of action of NTZ may probably be explained by the ability of the enzyme azoreductase of several bacterial species to reduce nitroaromatic drugs. The disinfectant activity of NTZ promotes healing in the treatment of wounds produced by trauma, burn or surgery procedures. This pharmaceutical compound can also be utilized in the treatment of bacterial contaminations caused by rejection or epidemic nosocomial infections, piodermic infections and skin ulcers. NTZ is effective against a significant number of microorganisms, including *Staphylococcus aureus* and *Escherichia coli*, but however does not possess significant activity against

Pseudomonas aeruginosa, *Proteus mirabilis* and *Serratia marcescens* [2]. Considering the cutaneous route of administration and the release of active substances on the epidermis, in many cases, there is an interest to maximize the residence time of the compound on the skin, minimizing its transdermal absorption. Among the structures used for the controlled release of drugs, one may find liposomes, microparticles (microcapsules and microspheres) and nanoparticles (nanospheres and nanocapsules) [3, 4]. Microencapsulation is a process by which solids, liquids and even gases may be captured within microscopic particles through the formation of a thin layer around the substance. The capsules formed may release the content in a controlled fashion and under specific conditions. In the pharmaceutical industry, the microencapsulation technology has been applied for masking odors or flavors, conversion of liquids into solids, protection against atmospheric (abiotic) agents (humidity, light, heat and/or oxidation), reduction or elimination of gastric irritation or secondary effects caused by certain drugs, reduction of volatility, administration of incompatible drugs, improvement of the flow characteristics of powders, facilitation of the handling of toxic substances, aid in the dispersion of water-insoluble substances in aqueous media, among other functions [5, 6]. There is a wide variety of methods for pre-

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paring polymeric microspheres and microcapsules, which allows the incorporation of water soluble or fat soluble drugs. The selection of these techniques is dependent on the application to be given to the microcapsule, on the size desired, on the release mechanism and the physicochemical properties of both the active material and the encapsulating agent. Adding to this, for choosing the most appropriate method one should also consider the simplicity, reproducibility and feasibility of scaling [4, 5]. The main techniques used for the placement of active ingredients include mechanical, physical and chemical methods. Additionally, various polymers can also be used in the production of microparticles and their choice depends on the application requirements [7]. In the method involving physicochemical extrusion/solidification, the core material in liquid form, molten or in solution, is forced through an orifice (a thin tube or syringe) to form microdroplets whose size will be dependent on the diameter of the orifice and the exit speed of the material. The solidification of the coating material can occur by evaporation of the solvent, diffusion of the solvent or by chemical reaction. The extrusion method with ionotropic crosslinking is widely used in the encapsulation of drugs, being based on the reaction between divalent cations and some polymers with formation of interchain junctions (crosslinked) to obtain hydrogels [7, 8]. As encapsulating agents there is a wide range of choices of raw materials. Among them, one can mention alginates, which are polysaccharides derived from alginic acid, which in turn is obtained mainly from seaweed of the species *Laminaria*. This biopolymer belongs to the class of binary copolymers, containing $\beta(1 \rightarrow 4)$ bonds of D-manuronic (M) acid and α -L-guluronic (G) acid, with wide variation in composition and sequential structure. Alginates possess unique colloidal properties such as natural compression, stabilization, suspension, film-forming, emulsion and gel formation. Alginates also have a unique feature, which is its willingness to react with polyvalent cations, especially calcium ions, thus resulting in stronger gels or insoluble polymers [9, 10]. The major goal of the research effort entertained herein was to prepare and characterize calcium alginate microparticles encapsulating NTZ, with concomitant use in the preparation of pharmaceutical formulations in the form of gels for topical applications.

2. MATERIAL AND METHODS

2.1. Reagents

All reagents utilized in this research work were of pharmaceutical purity or PA grade, and were used without further purification. The water used was purified in a Milli-Q Elga Purelab system (Molsheim, France) to a final conductivity of ca. $18.2 \text{ M}\Omega \cdot \text{cm}^{-1}$. Sodium alginate (low viscosity) 90.8%-106.0% dry weight (w/w), lot 1000896, and sodium alginate (high viscosity) 90.8%-106.0% dry weight (w/w), lot 0805920, were purchased from Vetec Química fina Ltda (Rio de Janeiro, Brazil); Anhydrous calcium chloride (powder, PA, lot 07060931) was purchased from CAQ - Casa da Química (Diadema, São Paulo, Brazil); Nitrofurazone (C.A.S. 59-87-0, molecular formula $\text{C}_6\text{H}_6\text{N}_4\text{O}_4$) 97.3% pure (dry weight basis, w/w), of chinese origin, was a kind gift by Henrifarma Produtos Químicos e Farmacêuticos Ltda (São Paulo, Brazil). Polyethyleneglycol 300 (lot 110217C914C2) was purchased

from Labsynter - Produtos para Laboratório Ltda (Diadema, São Paulo, Brazil). BHI nutritive broth (Brain Heart Infusion) was purchased from Fluka (St. Louis MO, U.S.A.). Mannitol Salt Agar was purchased from Prodimol Biotecnologia/SA (São Paulo SP, Brazil).

2.2. Biological Material

The microbial strains used were a kind gift from the Microbiology Laboratory of UBM (University Centre of Barra Mansa/RJ, Brazil), consisting in a strain of *Escherichia coli* (ATCC 25922, beta-lactamase negative), a strain of *Pseudomonas aeruginosa* (ATCC 27853), and a strain of *Staphylococcus aureus* (ATCC 250923, susceptible to oxacillin and penicillin).

2.3. Experimental procedures

2.3.1. Preparation of Microparticles Containing Encapsulated NTZ

Microparticles containing encapsulated NTZ were prepared by extrusion/solidification, via ionotropic gelification of sodium alginate in a three-step sequential procedure. The final formulation can be found in Table 1. In **step I**, (preparation of the nitrofurazone and sodium alginate solution), one has determined initially the best proportion between low- and high-viscosities sodium alginate. For this, the percent relationships 40:60, 50:50 and 60:40 of low-viscosity and high-viscosity sodium alginates, respectively, were tested. Nitrofurazone and sodium alginate were weighed separately, and 95 mL of ultrapure water were heated up to 60°C. The heated ultrapure water was then added slowly to the sodium alginate until its full dissolution, under magnetic stirring during ca. 10 min, using a magnetic stirring device from Fisaton (model 752 A, São Paulo, Brazil). Following formation of an homogeneous alginate gel, powder nitrofurazone was then added under manual stirring during ca. 2 min. In **step II** (extrusion), the homogeneous dispersion produced in **step I** was allowed to stand at rest during ca. 30 min until attaining room temperature (25°C), after which it was transferred into the reservoir of a compressed-air painting gun from Mac Loren (model P-100, Garça, Brazil). The sodium alginate-NTZ dispersion, under pressure, was then sprayed over a calcium chloride solution (1.5%, w/w) at room temperature (25°C), thus producing calcium alginate microparticles that remained scattered within the calcium chloride solution. Following their production, the microparticles were filtered and placed into another calcium chloride (2%, w/w) solution for an extra 30 min. In **step III** (drying), the microparticles produced were filtered in a sieve from Ber Tel (mesh of 180 mm, Caieiras, Brazil), and were allowed to dry at room temperature (25°C) during 24 h in the absence of light.

2.3.2. Physico-chemical Characterization of the Microparticles Containing Encapsulated Nitrofurazone

The microparticles containing encapsulated nitrofurazone were characterized, physico-chemically, via (i) evaluation of their microstructural morphology using scanning electron microscopy, (ii) determination of the amount of drug associated to the microparticles by UV-Vis spectrophotometry, (iii)

study of the NTZ association pattern to the reactants utilized to produce the microparticles, via both thermal analyses (TGA and DSC) and infrared spectrophotometry with Fourier transform (FTIR), and (iv) rheology studies.

Table 1. Composition of the calcium alginate microparticles with encapsulated nitrofurazone, produced via the extrusion/solidification technique.

| Component | Concentration (%, w/w) |
|---|---------------------------|
| 60:40 (% w/w) of high viscosity sodium alginate and low viscosity sodium alginate | 2.0 |
| Nitrofurazone | 0.2 |
| Calcium chloride | 2.0 |
| Ultrapure water | Sat 100% |

Notes: Sat – Sufficient amount to.

2.3.2.1. Structural Microanalysis of the Microparticles Containing Encapsulated Nitrofurazone via SEM

The surface of the microparticles containing encapsulated nitrofurazone was duly observed in a scanning electron microscope (from JEOL, model JSM-63660 CV, Tokyo, Japan). Microparticle samples were sputter coated with colloidal gold under vacuum, and placed in the microscope chamber. Microphotographs were gathered using electron beams with acceleration speeds of 10-20 keV. The samples were randomly scanned and photomicrographed at magnifications of x200 up to x300.

2.3.2.2. Thermal Analyses (TGA and DSC)

The thermogravimetric characterization of the microparticles was accomplished via thermogravimetric analysis (TGA) whereas the thermal analyses was pursued by differential scanning calorimetry (DSC). The TGA analyses were carried out using a thermogravimeter from TA Instruments (model 2050, New Castle, U.S.A.). For the DSC analyses one has used a differential scanning microcalorimeter from TA Instruments (model MDSC 2910, New Castle, U.S.A.), with the microparticle samples being subjected to a linear temperature increase from ca. 20°C up to 300°C, at a constant heating rate of 10°C min⁻¹.

2.3.2.3. Analyses via Infrared Spectrophotometry with Fourier Transform

The infrared spectra of both pure nitrofurazone and samples of microparticles containing encapsulated nitrofurazone were gathered in the range of wave number from 4000 to 100 cm⁻¹ during 64 scans, with a resolution of 4 cm⁻¹, using an infrared spectrophotometer (Illuminat IR II from Smiths, Watfords, England) with Fourier transform. FT-IR spectra were obtained using an infrared microprobe (Illuminat IR, model II, from Smiths, Watfords, England), coupled with an optical microscope (from Olympus, model BX51, Tokyo, Japan).

2.3.2.4. Determination of the Nitrofurazone Content of the Microparticles and of the Encapsulation Efficiency

The amount of nitrofurazone incorporated within the microparticles was calculated by the difference between the total concentration of NTZ offered to produce the dispersion of NTZ-sodium alginate and the concentration of free NTZ in the supernatant (calcium chloride solution) after the encapsulation process. The amounts of NTZ were determined spectrophotometrically in the UV-Vis at a wavelength of 375 nm [11] using a Multispec spectrophotometer from Shimadzu (model 1501, Kyoto, Japan).

2.3.3. Preparation of the Pharmaceutical Formulation in the Form of a Gel for Topical Application

For the preparation of the pharmaceutical formulation in the form of a gel, all components were weighed separately. 90 mL of ultrapure water were heated up to ca. 60°C, in a 250 mL beaker, and Aristoflex[®] was slowly added under manual stirring until obtention of a translucent and viscous gel. Slowly and with constant manual stirring, the remaining components were also added (propyleneglycol, polyethyleneglycol), as well as the preservative mixture (phenoxyethanol, methylparaben, propylparaben). After the gel preparation was complete, it was left standing for ca. 30 min until attaining room temperature (25°C). Next, the microparticles containing encapsulated nitrofurazone were duly added to the gel and the resulting preparation was thoroughly and carefully homogenized so as to fully disperse the microparticles in the gel. To evaluate the physico-chemical characteristics of the gel containing microparticles with encapsulated nitrofurazone, two gels were prepared, one without microparticles and another with free nitrofurazone, according to the same aforementioned procedure. The formulations produced are detailed in Table 2.

2.3.4. Characterization of the Gel Integrating Microparticles with Encapsulated Nitrofurazone

The gel integrating microparticles with encapsulated nitrofurazone was characterized from the point of view of its viscosity, content of active principle, photostability and antimicrobial activity, according to the procedures described next.

2.3.4.1. Photostability Assays

The gel photostability assays were performed according to the procedures described by Melo *et al.* [12]. As light source, a UV lamp was used, placed inside a chamber with dimensions 20cm x 27cm x 23cm, with the gel sample being irradiated at a distance of 18 cm from the lamp. The photostability assays were carried out by exposing ca. 10 g of the gels in Petri dishes isolated with polyethylene film (to avoid water evaporation and concomitant mass losses). The photostability assays were performed in samples of Aristoflex[®] AVC (control sample), Aristoflex[®] AVC gel integrating free nitrofurazone, and Aristoflex[®] AVC gel integrating microparticles with encapsulated nitrofurazone. To determine the content in nitrofurazone and its relationship with photostability, samples were withdrawn at time intervals of 60 min during a total timeframe of 3 h of UV exposure. After this time period, all samples were maintained under UV light for

Table 2. Formulations developed to produce the gels with calcium alginate microparticles with encapsulated nitrofurazone, plain gels without any microparticles, and gels with added free (pure) nitrofurazone.

| Formulation component | | Concentration (% , w/w) | | |
|---|----------------|---|-----------------------------------|---|
| | | Gel with embedded calcium alginate microparticles with encapsulated nitrofurazone | Gel with added free nitrofurazone | Gel without calcium alginate microparticles with encapsulated nitrofurazone |
| Aristoflex® AVC | | 4.0% | 4.0% | 4.0% |
| Calcium alginate microparticles with encapsulated nitrofurazone | | 0.2% | ----- | ----- |
| Propyleneglycol | | 5.0% | 5.0% | 5.0% |
| (free) Nitrofurazone | | ----- | 0.2% | ----- |
| Preservative solution | Phenoxyethanol | 0.4% | 0.4% | 0.4% |
| | Methylparaben | 0.15% | 0.15% | 0.15% |
| | Propylparaben | 0.02% | 0.02% | 0.02% |
| Ultrapure water | | *Sat 100% | *Sat 100% | *Sat 100% |

Notes: Sat – quantidade suficiente para.

24 h. The amount of nitrofurazone in the samples was determined by UV-Vis spectrophotometry at a wavelength of 375 nm [11].

2.3.4.2. Rheological Characterization of the Gels Produced

The viscosity of the several gels produced was determined using a rheometer from Brookfield (model Dv I Prime, U.S.A.). For the gel samples containing microparticles with encapsulated nitrofurazone, Brookfield *spindles* with references S-05, S-06 and S-07 were used, at a rotation speed of 10 rpm and at room temperature (25°C). All analyses were performed in triplicate. Besides viscosity values, the rheometer software also provided shear stress data as a function of shear rate, and therefore this information was also used for the rheological characterization of the samples of the various gels produced.

2.3.4.3. Determination of Nitrofurazone Content by UV-Vis Spectrophotometry

The methodology utilized for determination of the nitrofurazone content was the one developed by [11] involving UV-Vis spectrophotometry, using polyethyleneglycol to dissolve nitrofurazone, with absorbance readings at a wavelength of 375 nm.

2.3.4.4. Microbiological Assays via the Agar Diffusion Method

The antimicrobial efficacy of the gel integrating microparticles with encapsulated nitrofurazone was determined by applying the agar diffusion technique described by Bauer *et al.* [13] and Jorgensen *et al.* [14], as well as according to the standards of the Clinical and Laboratory Standards Institute [15]. The assays were carried out using a strain of *Staphylococcus aureus* (CCCD S007 - Cefar Diagnóstico Ltda, São Paulo, Brazil). The *Staphylococcus aureus* strain was, initially, inoculated in BHI nutritive broth (Brain Heart

Infusion) and maintained at 37°C ± 0.5°C during 24 h, to allow growth of the bacteria. Following this time period, the bacterial culture was inoculated into grooves on two Petri dishes containing Mannitol Salt Agar as culture medium. The samples to be tested were then applied on the inoculated medium, in duplicate: **quadrant 1** - gel optimized with 4% (w/w) ARISTOFLEX®, without microparticles with encapsulated nitrofurazone (negative inhibition standard); **quadrant 2** - optimized gel with 4% (w/w) ARISTOFLEX® integrating microparticles with encapsulated nitrofurazone; **quadrant 3** - sterile filter paper disc, impregnated with a 0.2% (w/v) solution of nitrofurazone in propyleneglycol (positive inhibition standard); and **quadrant 4** - sterile filter paper disc, impregnated with propyleneglycol (negative inhibition standard). Propyleneglycol and the solution of nitrofurazone in propyleneglycol were impregnated in sterile filter paper discs 7.0 mm in diameter, and inoculated in the Petri dishes with the aid of sterile tweezers near a Bunsen burner flame. The gel samples were inoculated in the Petri dishes with the aid of a sterile loop. The Petri dishes were then inverted and incubated under aerobic conditions at 37°C ± 0.5°C during 48 h. After this time period, the plates were visually inspected for observation (or not) of any growth inhibition halos.

3. RESULTS AND DISCUSSION

3.1. Production of Calcium Alginate Microparticles with Encapsulated Nitrofurazone

For the production of calcium alginate microparticles, the extrusion/solidification technique was applied. Sodium alginate is extensively used as encapsulating material, since it is a natural polymer of relatively low cost and easily obtainable, biocompatible, non-toxic and biodegradable. Having these features in mind, sodium alginate has been used to prepare microparticles containing different active principles and in different processes [10, 16,]. The extrusion/solidification

technique requires that the drug is added to a dispersion of sodium alginate and then this dispersion is extruded, with the particles being collected in a calcium chloride solution. The extrusion/solidification process using alginate occurs via ionotropic gelation of sodium alginate. The ionotropic gelation of sodium alginate occurs upon binding of divalent calcium ions to blocks of guluronic acid from the alginate chains, constituting the so-called "egg-box" model [17]. To obtain particles with uniform size, with maintenance of their shape after drying, the tests were focused in the definition of the most appropriate sodium alginate, by using low- and high-viscosity sodium alginate in different mass proportions. The best mass proportion found was 60:40 of high-viscosity sodium alginate and low-viscosity sodium alginate, respectively. The microparticles produced with this proportion of high- and low-viscosity sodium alginates were homogeneous in size and presented an adequate resistance. Regarding the extrusion process, a positive-pressure painting gun was found to be the most adequate method, producing microparticles with uniform conformations and reduced size.

3.2. Physico-chemical Characterization of the Particles Produced

3.2.1. SEM Analyses

The particles produced were fully characterized with respect to their morphology via scanning electron microscopy (SEM), nitrofurazone encapsulation efficiency, infrared spectrophotometry with Fourier transform, and thermal analyses via both TGA and DSC. The SEM analyses revealed particles with sizes between 500 and 800 μm and with a slightly oval shape (Fig. 1). The particles produced can, therefore, be characterized as microparticles, since the spectrum of sizes for microparticles varies between 1 and 1000 μm [18, 19]. According to Wang *et al.* [20], the concentration of sodium alginate may influence the particle shape and, usually, a mass concentration of sodium alginate between 0.8 and 1.5% is used to produce microparticles. These authors also consider that, when the percentage of sodium alginate is higher than the values cited the particles may present an oval shape. This was in fact observed in the present research effort, since the formulation optimized integrated 2.0 % (w/w) of sodium alginate. Naturally, beyond polymer concentration, the shape of the particles can also be strongly influenced by the extrusion process employed, temperature and feeding rate to the extruder device, amongst other factors.

3.2.2. FTIR Analyses

In (Fig. 2), one can see the infrared spectra of the calcium alginate microparticles with encapsulated nitrofurazone (Fig. 2a), of the calcium alginate microparticles without nitrofurazone (see Fig. 2b), and of pure nitrofurazone (Fig. 2c). In the infrared spectra of the calcium alginate microparticles, with and without encapsulated nitrofurazone, one can observe a peak in the wave number close to the region of 1590 cm^{-1} , probably indicating the presence of calcium ions [21]. The peak in the wavelength region of 3500 cm^{-1} , characteristic of the stretching of the OH and bond with water [22], is observed in all spectra. By comparing the infrared spectra of the calcium alginate microparticles with and without encapsulated nitrofurazone, one can see that there is no displace-

ment of the main peaks, only a change in intensity. This may indicate that nitrofurazone has not established a strong connection with the polymer.

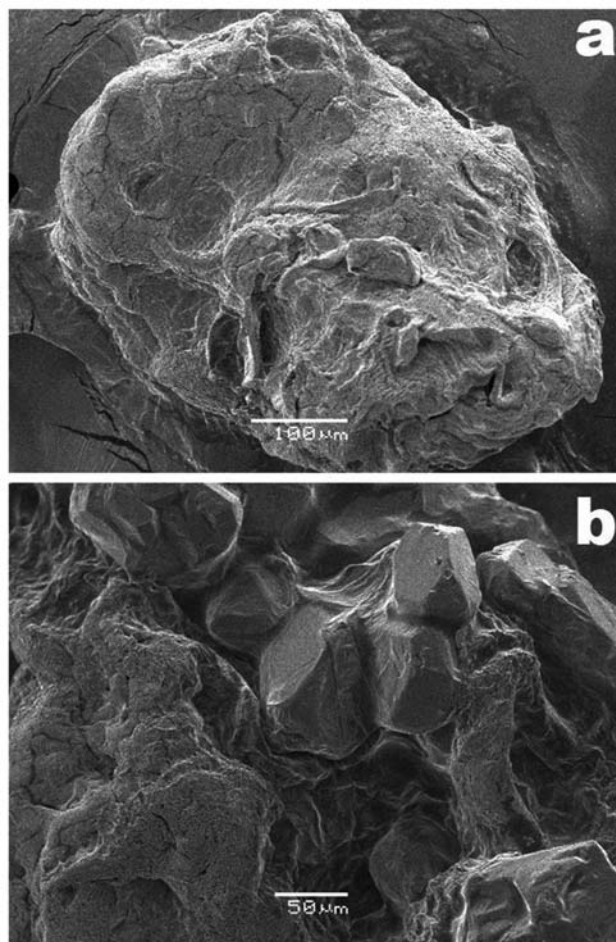


Fig. (1). Scanning electron microphotograph of a calcium alginate microparticle with encapsulated nitrofurazone, at different magnifications: (a) x200; (b) x300.

3.2.3. Encapsulation Efficiency

The efficacy of encapsulation is the ratio between the amount of encapsulated substance in the microparticles and the (total) amount of theoretical active employed. The encapsulation efficiency of active principles encountered in the specialty literature varies widely. Soni *et al.* [23] point, generally, to an encapsulation efficiency in the magnitude of 70-93%. However, according to Schaffazick *et al.* [24], this range of encapsulation efficiencies can vary even more, indicating association rates of some drugs from 39% to 100%. The encapsulation efficiency produced in the present research effort was of $97.8\% \pm 1.1\%$, a value which is in good agreement with those reported by Honary *et al.* [25] for the encapsulation efficiency of prednisone in microparticles of alginate and chitosan, viz. from $98.97\% \pm 0.97\%$ to $96.68\% \pm 0.40\%$. The rate of encapsulation varies as a function of several parameters, viz. the process utilized to produce the nano/microparticles, the type of drug selected, type and concentration of the polymer employed, rate of solvent elimination, solubility of the polymer in the solvent, amongst other factors [24]. Hence, the values for the encapsulation effi-

ciency produced in the research effort entertained herein can be considered as very good.

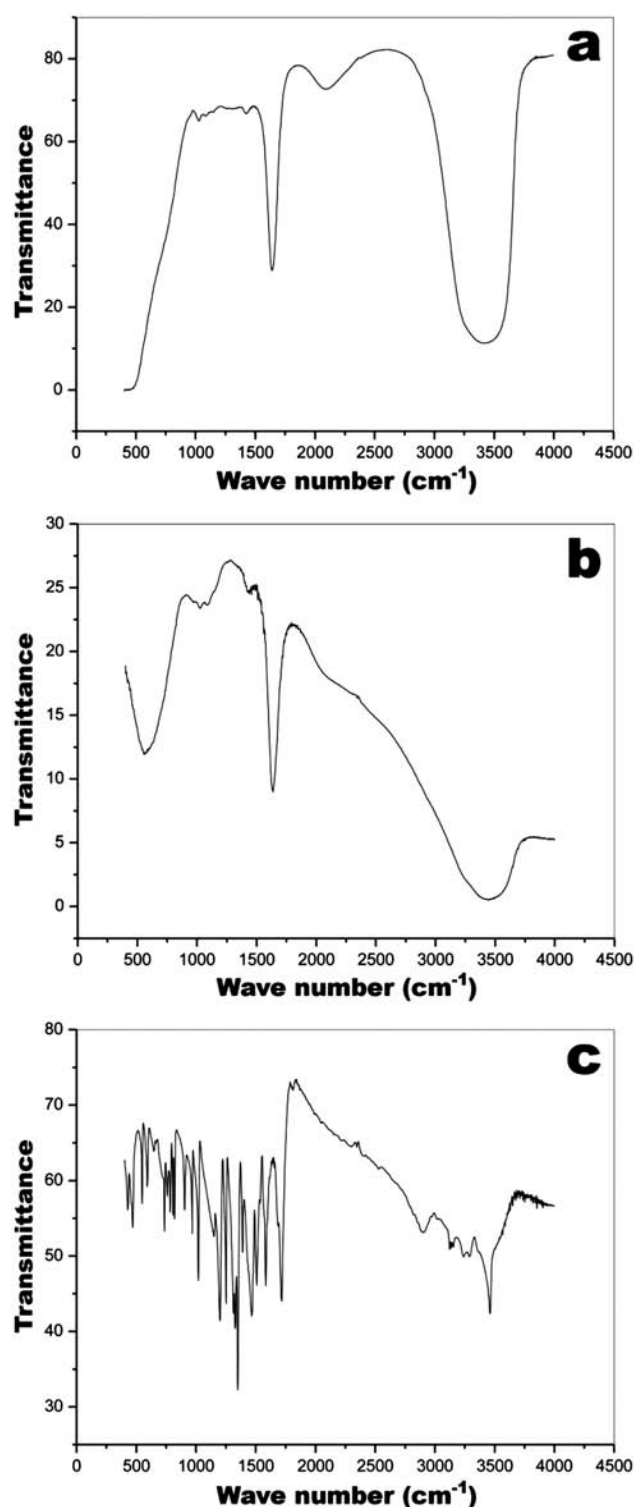


Fig. (2). Infrared spectra of (a) calcium alginate microparticles with encapsulated nitrofurazone, of (b) plain calcium alginate microparticles without nitrofurazone, and of (c) pure nitrofurazone. [Processing parameters: Image (magnification, x36.0; width, 252.1 μm ; height, 189.0 μm); experimental (analysis scans, 64; background scans, 64; resolution of 4 cm^{-1} ; spectral range, 4000.0-100.0 cm^{-1} ; detector type, MCT; objective, 36X-ATR)].

3.2.4. Thermal Analyses

The DSC analyses of calcium alginate microparticles without encapsulated nitrofurazone (see Fig. 3b) allows observation of a major endothermal event (melting) at 212.62°C. Regarding the calcium alginate microparticles with encapsulated nitrofurazone (Fig. 3a), the DSC analyses produced a melting temperature of 213.40°C. When comparing these temperatures a slight shift can be observed, which may potentially indicate that nitrofurazone was not strongly bound to the polymer.

The TGA curve of the microparticles with encapsulated nitrofurazone (Fig. 4a) showed three significant thermal events, with the first thermal event with loss of water occurring from 58.40°C to 89.00°C (78.31% mass loss). The second thermal event occurred at 192.46°C, and may probably indicate the formation of calcium carbonate (1.69% mass loss), whereas the third thermal event which may have accounted for the carbonization of the polymer chains occurred in the range of 286.87°C to 640.80°C (1.045% plus 1.050% mass losses). The analysis of the TGA curve of the calcium alginate microparticles without encapsulated nitrofurazone (Fig. 4b) indicated a significant mass loss (42.12%) starting at 55.94°C, probably due to the elimination of water. At 291.16°C, a 16.58% mass loss occurred, probably due to carbonization of the polymer chains. Hence, one can infer that when compared to the plain calcium alginate microparticles, the calcium alginate microparticles with encapsulated nitrofurazone are more prone to decomposition.

3.3. Physico-chemical Characterization of the Gel Produced

Generally, the substances responsible for the formation of gels are polymers that, when dispersed in an aqueous medium, assume a conformation that confers increased viscosity to the formulation. The type and percentage of polymer used in the formulation can affect the rheological behavior of the pharmaceutical formulation and hence influence the physical stability of the product [26]. Therefore, for the quality control of the product developed one performed several assays to verify the stability of the product's viscosity upon storage, determined the drug content, and assessed the photostability and antimicrobial efficacy.

3.3.1. Rheological Analyses

In semi-solid dosage forms, the determination of rheological characteristics is fundamental in assessing the quality of the product and its consequent acceptance by the consumer. The rheological characteristics are important properties to be considered in the manufacture, storage and application of topical products, and can influence parameters such as the physical stability of the system, spreadability and sensory characteristics, as well as the purposes of use [26]. In the research effort entertained herein, gels produced at concentrations ranging from 2.5% (w/w) to 5.0% (w/w) of ARISTOFLEX[®] AVC were evaluated for viscosity, to define the best percentage of gelling agent. These analyses proved necessary because the incorporation of calcium alginate microparticles with encapsulated nitrofurazone caused a reduction in viscosity in the original formulation. The rheological behavior of semi-solid pharmaceutical forms can be evalu-

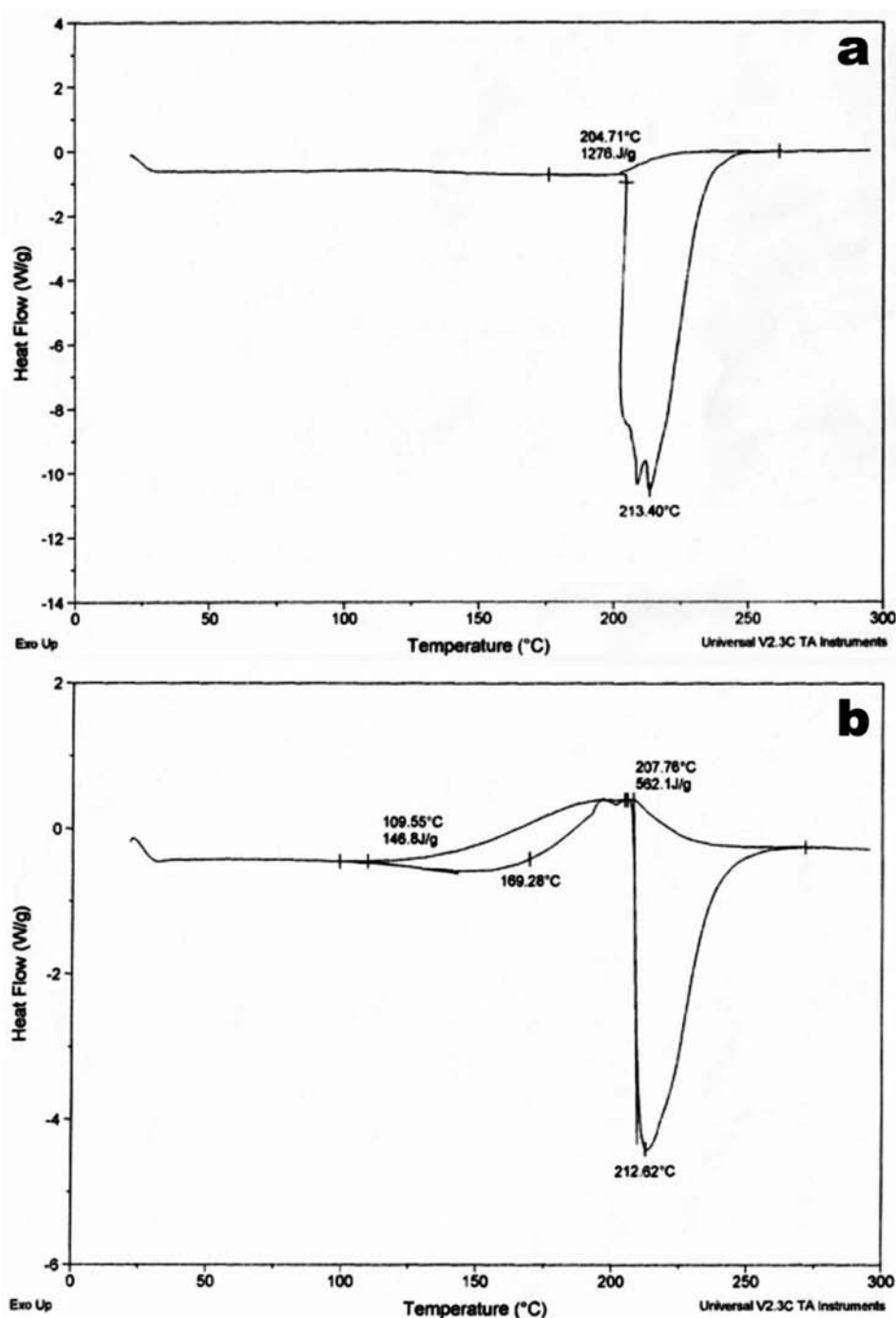


Fig. (3). Differential scanning calorimetry (DSC) analyses of (a) calcium alginate microparticles with encapsulated nitrofurazone and of (b) plain calcium alginate microparticles (without nitrofurazone).

ated via measurements of viscosity, using a viscosimeter. All analyses were performed in triplicate at 0, 30, 45, 60, 75 and 90 days of storage. From the results obtained (Fig. 5) it can be seen that the percentage of gellifying agent which resulted in the better outcome in relation to the formulation's viscosity was 4% (w/w) of ARISTOFLEX[®] AVC. During the 90 days of storage, the gel produced with 4% (w/w) ARISTOFLEX[®] AVC maintained a proper viscosity, thus maintaining the expected characteristics of a formulation for topical use. Hence, the gel produced with 4% (w/w) ARISTO-

FLEX[®] AVC was the formulation of choice for carrying the calcium alginate microparticles with encapsulated nitrofurazone.

The rheological behavior of the gel was also evaluated, being considered shear thinning since the apparent viscosity gradually decreases with increasing shear stress. The results obtained (Fig. 6) were characteristic of pseudoplastic fluid. The shear thinning behavior is more common among non-Newtonian fluid, also called shear-thinning and reflects a temporary change in the structure of molecules during shear.

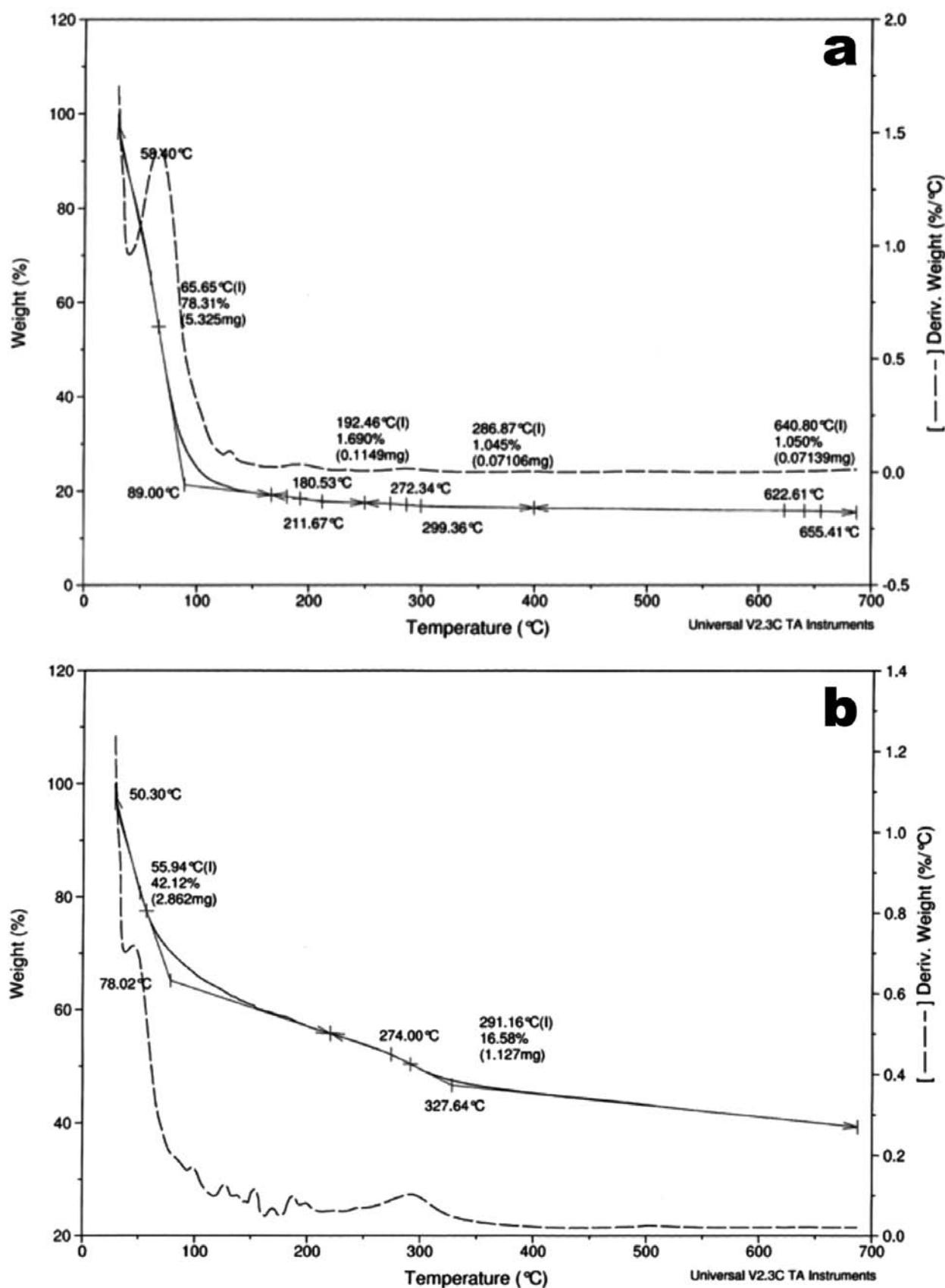


Fig. (4). Thermogravimetric curves (TGA) of (a) calcium alginate microparticles with encapsulated nitrofurazone and of (b) plain calcium alginate microparticles (without nitrofurazone).

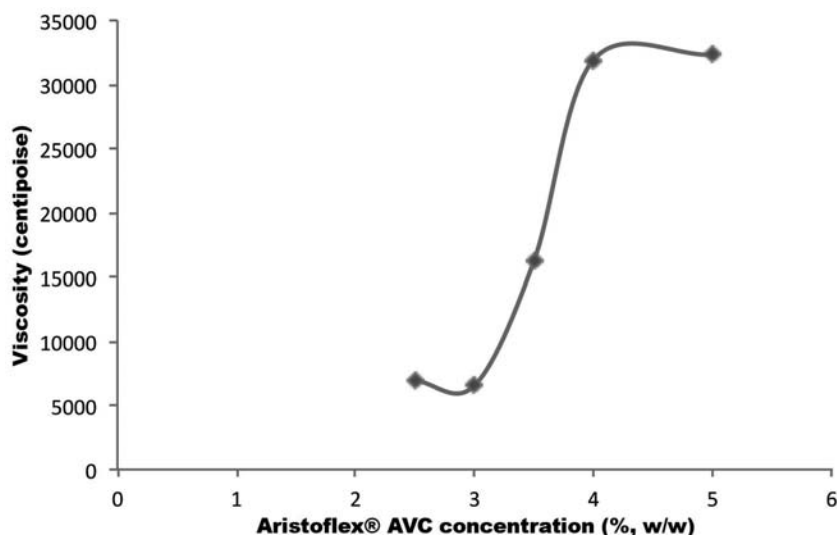


Fig. (5). Viscosity of the gel as a function of gellifying agent concentration (Aristoflex® AVC) (2.5; 3.0; 3.5; 4.0 and 5.0%, w/w), after a storage timeframe of 90 d.

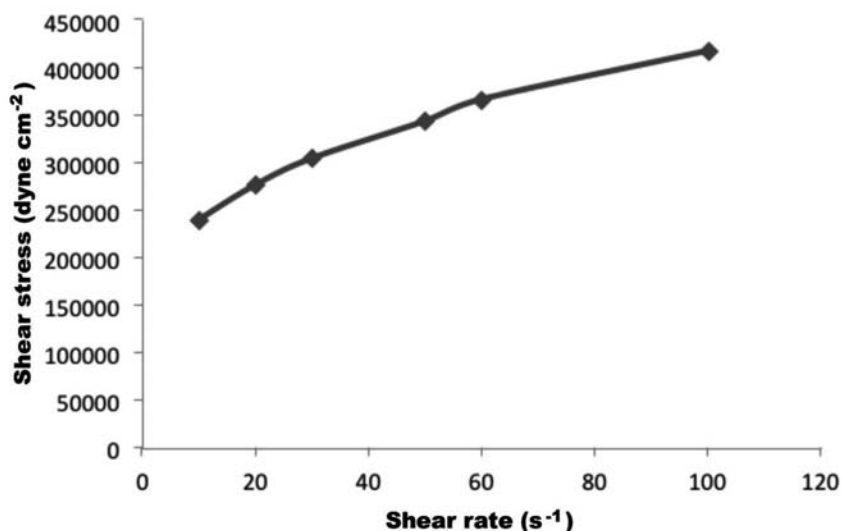


Fig. (6). Rheogram (shear stress as a function of shear rate) of the final gel formulation with 4% (w/w) Aristoflex® AVC, with embedded calcium alginate microparticles with encapsulated nitrofurazone.

3.3.2. Determination of NTZ Concentration via UV-Vis Spectrophotometry

For the determination of nitrofurazone concentration in the final gel formulation integrating calcium alginate microparticles with encapsulated nitrofurazone, the spectrophotometric methodology described by Tubino *et al.* [11] was utilized, with absorbance readings at 375 nm. Initially, one has verified the linearity of the spectrophotometric method, by using nitrofurazone solutions with concentrations ranging from 2.0 mg L⁻¹ to 20.0 mg L⁻¹, which produced the linear relationship $\text{Abs@375nm} = 0.089 \times [\text{NTZ, mg L}^{-1}] - 0.007$, with a correlation coefficient (R^2) equal to 0.999. The precision of the NTZ concentrations analyzed (2.5, 10, 12.5, 20 mg L⁻¹) exhibited a coefficient of variation between 0.27 and 0.47%, an accuracy between 99.87% and 109.36%. The quantification and detection limits were of 0.04 mg L⁻¹ and 0.01 mg L⁻¹, respectively, meaning that all parameters con-

sidered were adequate for the analysis proposed. Through the data gathered in the determination of nitrofurazone concentration in samples of gels containing calcium alginate microparticles with encapsulated nitrofurazone, one obtained a content in active drug of ca. $0.18\% \pm 0.05\%$, meaning that the final gel formulation contained 90% of the drug content offered to produce the formulation. The technique employed to prepare the gel formulation consisted in adding the microparticles to the gel. Perhaps, by using the suspension of microparticles itself as the aqueous phase in the preparation of the gel, one could maintain the nitrofurazone concentration of 0.2% (w/w), considered as appropriate for the active drug used.

3.3.3. Photostability Assays

Nitrofurazone is a drug that, when exposed to sun light, undergoes isomerization with formation of red-yellowish

compounds. The photodegradation process of nitrofurazone may be explained by a kinetic model involving three first-order reactions [27]. To ensure a constant effectiveness for the developed formulations, the gels were subjected to UV light irradiation in a UV light chamber, according to the procedure described by Melo *et al.* [12], and the levels of active drug were assessed via spectrophotometry. For this purpose, analyzes were performed on the gels produced with ARISTOFLEX[®] AVC at the concentrations of 2.5%, 3.0%, 3.5%, 4.0%, and 5.0% (w/w) and integrating microparticles with encapsulated nitrofurazone. For the purpose of comparison of the results, one subjected to the same conditions a gel of ARISTOFLEX[®] AVC added with free nitrofurazone and a gel of ARISTOFLEX[®] AVC without addition of either microparticles containing encapsulated nitrofurazone or free nitrofurazone. Table 3 presents the results encountered for the concentration of nitrofurazone following exposure of the gel samples to UV light radiation after 1 h, 2 h, 3 h and 24 h of exposure. After 3 h of exposure to UV radiation, there was no significative variation in the produced contents of nitrofurazone, and thus one opted to determine the nitrofurazone content after 24 h of exposure to UV radiation. It can be seen that, after 24 h of exposure, the amount of nitrofurazone exhibited higher values in all formulations. This is an indication that there was nitrofurazone degradation in all formulations, since the spectrophotometric method employed does not allow differentiation between the active drug and its degradation products. It can be concluded, therefore, that the topical product developed requires protection from light so as to maintain its integrity.

3.3.4. Anti-microbial Assays

Staphylococcus aureus was the pathogenic microorganism chosen to test the antimicrobial activity of optimized gel incorporating calcium alginate microparticles containing encapsulated nitrofurazone. Since this is an opportunistic pathogen, *S. aureus* is one of the main responsables for infections in burn and surgical wounds [28]. Additionally, *S. aureus* is susceptible to the antimicrobial action of nitrofurazone.

zone when in doses greater than or equal to 4 $\mu\text{g mL}^{-1}$, according to Yilmaz *et al.* [29]. From a close inspection of (Fig. 7), one can notice that microparticles are capable of releasing the encapsulated nitrofurazone, which may allow the use of calcium alginate microparticles containing encapsulated nitrofurazone in pharmaceutical formulations for topical use. In fact, a careful inspection of (Fig. 7) (quadrant 2) reveals that application of the gel containing the microparticles with encapsulated nitrofurazone inhibits microbial growth (unlike the gel without incorporated microparticles, see quadrant 1 in Fig. 7), while the disk impregnated with 0.2% (w/v) nitrofurazone in propylene glycol produced a clear halo of inhibition of bacterial growth.

To eliminate any possibility of antimicrobial action of the solvent used for nitrofurazone, a sterile disk was impregnated with propylene glycol and also applied on the inoculated culture, and the result was clearly negative in terms of inhibition of bacterial growth (quadrant 4 in Fig. 7). Hence, the results clearly demonstrate the antimicrobial activity of the gel produced in this research effort, which integrated calcium alginate microparticles containing encapsulated nitrofurazone.

CONCLUSIONS

The system used to obtain calcium alginate microparticles at the lab scale proved to be effective, producing a high encapsulation efficiency of the drug. The physico-chemical analyses performed indicated microparticles with uniform size and with little interaction with the polymer employed in the process. These characteristics are appropriate to ensure the functionality of this type of drug release system. Incorporation of the calcium alginate microparticles into the initial gel formulation caused its destabilization, and thus a resetting of the content of gelling agent was required. After modification of the initial formulation, the gel presented stable viscosity and a good appearance. The gel embedded with calcium alginate microparticles containing encapsulated nitrofurazone also exhibited an appropriate antimicrobial activity for a product for topical use and drug content within ac-

Table 3. Nitrofurazone concentration in the gels after exposure to UV radiation, produced by a UV Mineralight Lamp - UVGL-58 (254nm).

| Gels produced with different concentrations (w/w) of Aristoflex [®] AVC | Nitrofurazone concentration (% w/w) as a function of exposure time to UV radiation | | | |
|--|--|--------|--------|--------|
| | 1 h | 2 h | 3 h | 24 h |
| 2.5 % * | 0.151 | 0.151 | 0.156 | 0.172 |
| 3.0 % * | 0.203 | 0.203 | 0.196 | 0.228 |
| 3.5 % * | 0.223 | 0.220 | 0.190 | 0.273 |
| 4.0 % * | 0.129 | 0.150 | 0.132 | 0.191 |
| 4.5 % * | 0.262 | 0.271 | 0.285 | 0.359 |
| 5.0 % * | 0.202 | 0.208 | 0.174 | 0.193 |
| 2.0% with nitrofurazone** | 0.1278 | 0.1309 | 0.1439 | 0.1711 |
| 2.0% without nitrofurazone*** | 0.0093 | 0.0093 | 0.0091 | 0.0097 |

Notes: *Gel with 0.2% (w/w) embedded calcium alginate microparticles with encapsulated nitrofurazone; **Gel produced with 2.0% (w/w) Aristoflex[®] AVC and 0.2% (w/w) free nitrofurazone; ***Gel produced with 2.0% (w/w) Aristoflex[®] AVC without nitrofurazone.

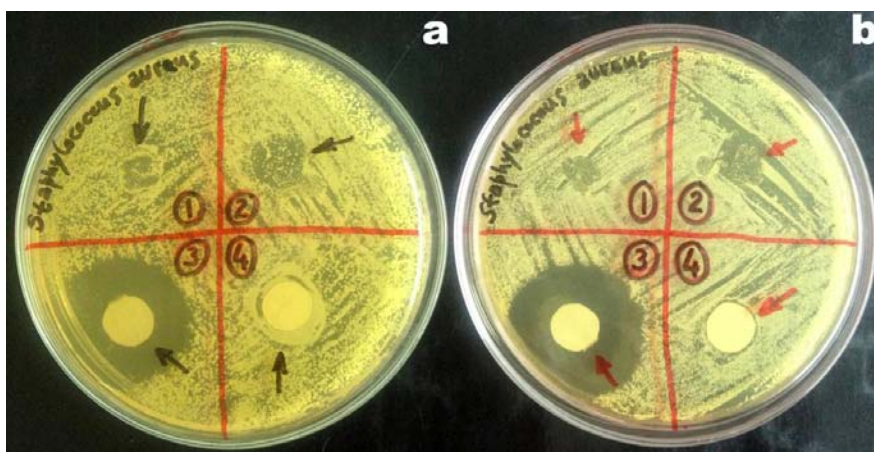


Fig. (7). Photographs of Petri dishes (two replicates) after performance of microbiological assays, displaying the results obtained. **Quadrant 1** - Plain optimized gel with 4% (w/w) gellifying Aristoflex® AVC, without calcium alginate microparticles with encapsulated nitrofurazone (negative standard of inhibition); **Quadrant 2** - Optimized gel with 4% (w/w) gellifying Aristoflex® AVC, with embedded calcium alginate microparticles with encapsulated nitrofurazone; **Quadrant 3** - Sterile filter paper disk, impregnated with 0.2% (w/v) nitrofurazone in propylene glycol (positive standard of inhibition); and **Quadrant 4** - Sterile filter paper disk, impregnated with propylene glycol (negative standard of inhibition); The arrows point to the place of application of the samples to be tested].

ceptable parameters. From the results obtained in the research effort entertained herein, the pharmaceutical dosage form proposed exhibited promising qualities for use as a topical antimicrobial gel.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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